SOME EXPERIMENTS ON THE EFFECTS OF HYPOPHYSECTOMY AND PITUITARY IMPLANTATIONS ON THE MALE FUNDULUS HETEROCLITUS [1, 2]

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While sufficient work has been accomplished to show that the hypophysis of fish secretes a gonadotropic principle, the hormonal relationships involved in the piscine sexual cyle are not clearly understood (compare Matthews, 1940 for a summary). For *Fundulus* Matthews (1939) found that after hypophysectomy, the testes failed to continue gametogenesis during the breeding period. The injection of mammalian pituitary extracts had no decisive effect upon the gonads. Matthews (1940) found, however, that the implantation of *Fundulus* pituitaries into non-hypophysectomized immature *Fundulus* induced gametogenic activity, especially in the male. The present investigations are complementary to those of Matthews. Positive effects from pituitary implantations were secured in hypophysectomized adult male *Fundulus*.

Effects of Hypophysectomy

On July 3–6, 73 freshly captured, mature male *Fundulus* were hypophysectomized. The opercular approach was used for this very simple operation. Control fish were given blank operations. All fish were maintained under identical conditions in running sea water, the temperature of which varied between 11° and 19° C. The water for the most part was near or below 15° C. The fish were fed almost daily on chopped clams. This diet appeared adequate, since the operated fish deposited fat as do fish in nature during the summer.

Mortality in operated fish was about 30 per cent. No significant difference was found between the mortality of hypophysectomized fish and those which received a blank operation. Over a two-month experimental period, the loss of the hypophysis seems to have little to do with the viability of *Fundulus*.

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[2] There is some doubt if this fish is strictly speaking, *Fundulus heteroclitus*. It may be a related heteroclitoid form.

At the time of hypophysectomy, the testes had passed their maximal development for the annual sexual cycle (Fig. 1). In nature, the maximal development occurring during the spring is followed by testicular regression. By late August, spermiogenetic transformations have almost ceased and the testes are practically devoid of sperm. As pointed out by Burger (1940), testicular regression does not occur as rapidly for fish kept in cool water (11°–17° C.) in the laboratory, as it does in the warmer water of the natural habitat. The degree of testicular regression attained by September 1, in fish which received blank operations, is shown in Fig. 2.

The effects on the testis after hypophysectomy were similar to those described by Matthews (1939) for other periods in the sexual cycle, viz., after complete testicular regression (October–December), and at the beginning of normal spermatogenesis (March–April).

The testes underwent a rapid reduction in size. The most obvious effect was a cessation of sperm formation. This cessation was not immediately a complete one. One month after hypophysectomy, however, only rare cysts of spermatids could be found. Two months after the operation, spermatids were absent in the six testes examined. At no time were spermatogonial multiplications suppressed. These divisions formed a well-defined cortical zone of spermatogonia. Cross-sections of testes from hypophysectomized fish are shown in Figs. 3 (August 2, one month after hypophysectomy), and 4 (September 1, two months after operation, cf. with control, Fig. 2). Thus it would appear that the loss of the hypophysis results in inhibition of spermatogenetic stages beyond those of spermatogonial division. Nevertheless,

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Fig. 1. Cross-section of a testis at time hypophysectomies were performed (July 3–6). This testis has passed its maximal sperm production. Black patches are sperm.

Fig. 2. Cross-section of a testis from a control fish, killed September 1; this fish has experienced a blank operation. Black patches are sperm. The cortical zone where spermatogenetic stages are visible, has become narrow (cf. Fig. 1).

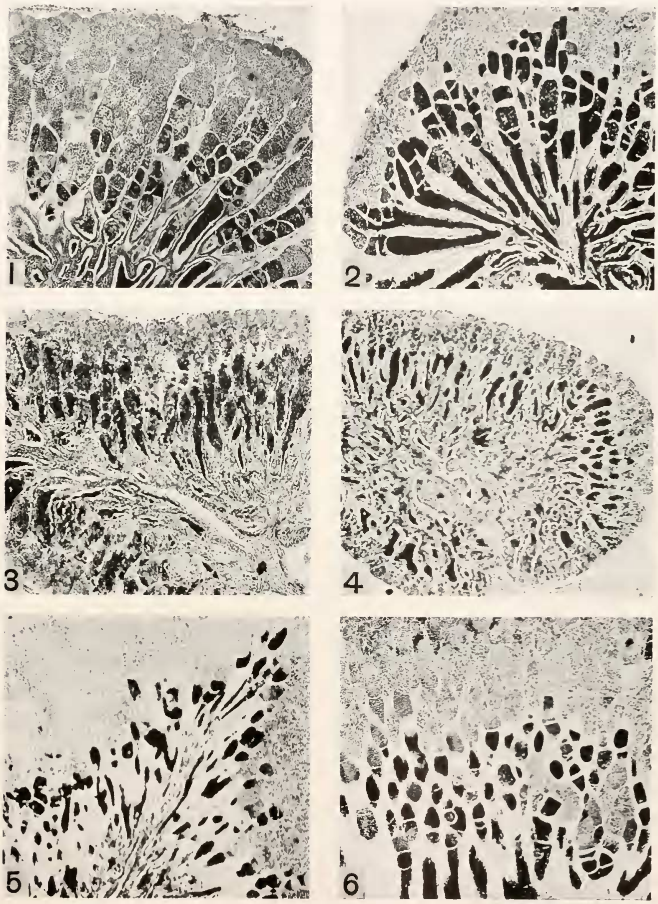
Fig. 3. Cross-section of a testis from a hypophysectomized fish, killed August 2. The cortical zone contains almost nothing but spermatogonia. Spermiogenesis has practically ceased.

Fig. 4. Cross-section of a testis from a hypophysectomized fish, killed September 2. The cortical zone of spermatogonia has grown more narrow (cf. Fig. 3, one month earlier).

Fig. 5. Cross-section of a hypophysectomized fish implanted with twenty pituitaries, beginning August 17 and killed September 2. The spermatogenetic zone has deepened noticeably. Newly-formed sperm are visible. Compare with control, Fig. 4.

Fig. 6. Cross-section of a testis from a fish which received a blank operation; this fish was implanted with twenty pituitaries beginning August 17 and killed September 2. Compare with control, Fig. 2.

it does seem true that once spermatogenesis has been initiated, spermiogenesis can continue for some time, and to a limited degree, in the absence of the pituitary. The fact that this spermatogenesis is not maintained in any great volume even for as long as one month, indicates,



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as Matthews (1939) has suggested, that the later stages of spermatogenesis are more sensitive to the absence of the pituitary than are the stages of spermatogonial division.

Effects of Pituitary Implantations

After the testes of the hypophysectomized fish had involuted sufficiently, both hypophysectomized fish and some operated controls were implanted with pituitaries from freshly captured, mature male *Fundulus*. The receptors selected were close to each other in size. The size of the donor fish was also roughly standardized. The implantations were made intraperitoneally. Ten hypophysectomized fish and three operated controls each received five fresh glands on each of the following days: August 17, 21, 26, and 29. These fish, which each received twenty glands, were sacrificed on September 2. Five hypophysectomized fish and two operated controls each received five fresh glands on each of the following days: August 21, 26, 29. These fish were killed on September 4. During this phase of the work, the water was almost constantly near 13° C.

The effects of the implantations were striking. Within five days after the first injections, the fish began to show pronounced sexual display antics. These peculiar swimming movements are common in fish during the breeding season and occur occasionally throughout the year in fish kept in aquaria. These movements were noticeably absent in the untreated hypophysectomized fish. By the tenth day after the first injection, the fish were in a frenzy of display.

Both series of implantations caused a recrudescence of the testes. After two weeks, the average weight of the testes of the fish which received twenty implants was slightly more than doubled, and the average volume was quadrupled, when compared with the average weight and volume of untreated hypophysectomized controls. The cortical zone of spermatogonia had deepened, while new transformations into spermatozoa were well established (Fig. 5). The hypophysectomized fish which each received fifteen glands likewise, formed new sperms. The weight and volume increases were the same as those for the fish which received twenty glands. The spermatogenetic stages were also the same. The non-hypophysectomized fish which were receptors of implants reacted as did the hypophysectomized receptors (Fig. 6). The control hypophysectomized fish (Fig. 4), and the control fish which experienced blank operations (Fig. 2) showed no testicular recrudescence during the experimental period.

These results clearly demonstrate that the pituitaries of adult male

Fundulus contain, and the testes of hypophysectomized and normal adult fish are responsive to, gonadotropic material. Matthews (1940) has shown that the immature testis of non-hypophysectomized *Fundulus* can be excited by Fundulus pituitaries implanted into the body cavity.

Discussion

The present study, together with that of Matthews (1940), permits an interpretation of the pituitary rhythm involved in the normal sexual cycle. During the period of sexual regression, pituitary secretion gradually declines. The pituitary does not abruptly cease to secrete, since active spermiogenesis in a gradually decreasing volume occurs during this regression. When the pituitary is removed, spermatogenesis is more quickly checked. In the late summer, pituitary secretion seems to be almost or entirely absent. Beginning in the fall and going through the winter, spermatogonial multiplications take place. The divisions can occur in the absence of the pituitary, but in the hypophysectomized fish there is no progressive increase in the number of these spermatogonia. Hence, the piling up in the testis of spermatogonia must be supported by hypophyseal secretion. The amount of this secretion is low, since very few spermatozoa are formed during the fall and winter. The spring spermatogenesis is accompanied by the highest phase of secretion. The gonadotropic material of the *Fundulus* pituitary is thus responsible for two phases of spermatogenesis: (1) the great volume of spermatogonial proliferation, and (2) maturation phenomena. It has been shown by Burger (1939) and Matthews (1939*a*) that the progressive phases at least of the sexual cycle of the male are influenced by the temperature of the water.

Summary

Adult male *Fundulus,* hypophysectomized shortly after maximal testicular development, show an inhibition of spermatogenesis for stages beyond those of spermatogonial multiplication. Spermatogonial divisions do not become numerous. The inhibition of the later stages is not immediately affected, since a few cysts continue to form sperm for as long as one month after hypophysectomy. These results confirm those of Matthews.

Both hypophysectomized adult male *Fundulus* and fish which received blank operations were maintained until sexual regression was well established. Implantations of twenty or fifteen pituitaries from normal male *Fundulus* caused within two weeks a recrudescence of the testes. Non-implanted controls showed none of this activity. It is concluded

that the pituitary of the adult male *Fundulus* contains gonadotropic material and that the testes of adult *Fundulus,* hypophysectomized or not, are responsive to this material.

The normal relation of the pituitary to the annual sexual cycle is discussed.

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